DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration

Memorandum

Date AUG 26 1997

From Deputy Director, Clinical and Review Policy, Office of Device Evaluation (HFZ-400), Center for Devices and Radiological Health (CDRH)

Subject Premarket Approval of Abbott Laboratories IMx® Tacrolimus II Assay

To The Director, CDRH
ORA _____

ISSUE. Publication of a notice announcing approval of the subject PMA.

FACTS. Tab A contains a FEDERAL REGISTER notice announcing:

(1) a premarket approval order for the above referenced medical device (Tab B); and

(2) the availability of a summary of safety and effectiveness data for the device (Tab C).

RECOMMENDATION. I recommend that the notice be signed and published.

Kimber C. Richter

Kimber C. Richter, M.D.

Attachments
Tab A - Notice
Tab B - Order
Tab C - S & E Summary

DECISION

Approved ✓ Disapproved _____ Date _____

Prepared by Tena Wei, CDRH, HFZ-440, 7-18-97, 594-1243

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

DRAFT

Food And Drug Administration

[DOCKET NO. _____]

Abbott Laboratories, Premarket Approval Of IMx® Tacrolimus II Assay

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing its approval of the application by Abbott Laboratories, Abbott Park, IL, for premarket approval, under the Federal Food, Drug, and Cosmetic Act (the act), of IMx® Tacrolimus II Assay. FDA's Center for Devices and Radiological Health (CDRH) notified the applicant, by letter of August 26, 1997, of the approval of the application.

DATES: Petitions for administrative review by (insert date 30 days after date of publication in the FEDERAL REGISTER).

ADDRESSES: Written requests for copies of the summary of safety and effectiveness data and petitions for administrative review to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Steven I. Gutman,

Center for Devices and Radiological Health (HFZ-440),

Food and Drug Administration,

2098 Gaither Road,

Rockville, MD 20850,

301-594-1243.

SUPPLEMENTARY INFORMATION: On February 18, 1997, Abbott Laboratories, Abbott Park, IL 60064-3527, submitted to CDRH an application for premarket approval of IMx® Tacrolimus II Assay. IMx® Tacrolimus II Assay is an in vitro reagent system for the quantitative determination of tacrolimus and some metabolites in human whole blood as an aid in the management of liver allograft patients receiving tacrolimus therapy.

In accordance with the provisions of section 515(c)(2) of the act (21 U.S.C. 360e(c)(2)) as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Clinical Chemistry and Toxicology Devices Panel of the Medical Devices Advisory Committee, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

On August 26, 1997, CDRH approved the application by a letter to the applicant from the Deputy Director, Clinical and Review Policy, of the Office of Device Evaluation, CDRH.

A summary of the safety and effectiveness data on which CDRH based its approval is on file in the Dockets Management Branch (address above) and is available from that office upon written request. Requests should be identified with the name of the device and the docket number found in brackets in the heading of this document.

Opportunity For Administrative Review

Section 515(d)(3) of the act authorizes any interested person to petition, under section 515(g) of the act, for administrative review of CDRH's decision to approve this application. A petitioner may request either a formal hearing under 21 CFR part 12 of FDA's administrative practices and procedures regulations or a review of the application and CDRH's action by an independent advisory committee of experts. A petition is to be in the form of a petition for reconsideration under 21 CFR 10.33(b). A petitioner shall identify the form of review requested (hearing or independent advisory committee) and shall submit with the petition supporting data and information showing that there is a genuine and substantial issue of material fact for resolution through administrative review. After reviewing the petition, FDA will decide whether to grant or deny the petition and will publish a notice of its decision in the FEDERAL REGISTER. If FDA grants the petition, the notice will state the issue to be reviewed, the form of the review to be used, the persons who may participate in the review, the time and place where the review will occur, and other details.

Petitioners may, at any time on or before (insert date 30 days after date of publication in the FEDERAL REGISTER), file with the Dockets Management Branch (address above) two copies of each petition and supporting data and information, identified with the name of the device and the docket number found in brackets in the heading of this document. Received petitions may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (secs. 515(d), 520(h) (21 U.S.C. 360e(d), 360j(h))) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.10) and redelegated to the Director, Center for Devices and Radiological Health (21 CFR 5.53).

Dated: _____.

_____

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Mr. Mark Littlefield
Senior Regulatory Specialist
Abbott Laboratories
ADD Regulatory Affairs, D-9V6 AP31
100 Abbott Park Road
Abbott Park, Illinois 60064-3537

AUG 26 1997

Re: P970007
IMx® Tacrolimus II Assay
Filed: February 18, 1997
Amended: May 13, June 10, July 21 and 31, 1997

Dear Mr. Littlefield:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the IMx® Tacrolimus II Assay. This device is indicated for use as an in-vitro reagent system for the quantitative determination of tacrolimus and some metabolites in human whole blood as an aid in the management of liver allograft patients receiving tacrolimus therapy. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter. Expiration dating for this device has been established and approved at 9 months at 2-8OC. This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(8).

The sale, distribution and use of this device are restricted to prescription use in accordance with 21 CFR 801.109.

CDRH will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

PMA Document Mail Center (HFZ-401)
Center for Devices and Radiological Health
Food and Drug Administration
9200 Corporate Blvd.
Rockville, Maryland 20850

If you have any questions concerning this approval order, please contact Alfred Montgomery, D.V.M., at (301) 594-1243.

Sincerely yours,

Kimber C. Richter

Kimber C. Richter, M.D.
Deputy Director, Clinical and
Review Policy
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

CONDITIONS OF APPROVAL

APPROVED LABELING. As soon as possible, and before commercial distribution of your device, submit three copies of an amendment to this PMA submission with copies of all approved labeling in final printed form to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration (FDA), 9200 Corporate Blvd., Rockville, Maryland 20850.

ADVERTISEMENT. No advertisement or other descriptive printed material issued by the applicant or private label distributor with respect to this device shall recommend or imply that the device may be used for any use that is not included in the FDA approved labeling for the device. If the FDA approval order has restricted the sale, distribution and use of the device to prescription use in accordance with 21 CFR 801.109 and specified that this restriction is being imposed in accordance with the provisions of section 520(e) of the act under the authority of section 515(d)(1)(B)(ii) of the act, all advertisements and other descriptive printed material issued by the applicant or distributor with respect to the device shall include a brief statement of the intended uses of the device and relevant warnings, precautions, side effects and contraindications.

PREMARKET APPROVAL APPLICATION (PMA) SUPPLEMENT. Before making any change affecting the safety or effectiveness of the device, submit a PMA supplement for review and approval by FDA unless the change is of a type for which a "Special PMA Supplement-Changes Being Effected" is permitted under 21 CFR 814.39(d) or an alternate submission is permitted in accordance with 21 CFR 814.39(e). A PMA supplement or alternate submission shall comply with applicable requirements under 21 CFR 814.39 of the final rule for Premarket Approval of Medical Devices.

All situations which require a PMA supplement cannot be briefly summarized, please consult the PMA regulation for further guidance. The guidance provided below is only for several key instances.

A PMA supplement must be submitted when unanticipated adverse effects, increases in the incidence of anticipated adverse effects, or device failures necessitate a labeling, manufacturing, or device modification.

A PMA supplement must be submitted if the device is to be modified and the modified device should be subjected to animal or laboratory or clinical testing designed to determine if the modified device remains safe and effective.

A "Special PMA Supplement - Changes Being Effected" is limited to the labeling, quality control and manufacturing process changes specified under 21 CFR 814.39(d)(2). It allows for the addition of, but not the replacement of previously approved, quality control specifications and test methods. These changes may be implemented before FDA approval upon acknowledgment by FDA that the submission is being processed as a "Special PMA Supplement - Changes Being Effected." This acknowledgment is in addition to that issued by the PMA Document Mail Center for all PMA supplements submitted. This procedure is not applicable to changes in device design, composition, specifications, circuitry, software or energy source.

Alternate submissions permitted under 21 CFR 814.39(e) apply to changes that otherwise require approval of a PMA supplement before implementation of the change and include the use of a 30-day PMA supplement or annual postapproval report. FDA must have previously indicated in an advisory opinion to the affected industry or in correspondence with the applicant that the alternate submission is permitted for the change. Before such can occur, FDA and the PMA applicant(s) involved must agree upon any needed testing protocol, test results, reporting format, information to be reported, and the alternate submission to be used.

POSTAPPROVAL REPORTS. Continued approval of this PMA is contingent upon the submission of postapproval reports required under 21 CFR 814.84 at intervals of 1 year from the date of approval of the original PMA. Postapproval reports for supplements approved under the original PMA, if applicable, are to be included in the next and subsequent annual reports for the original PMA unless specified otherwise in the approval order for the PMA supplement. Two copies identified as "Annual Report" and bearing the applicable PMA reference number are to be submitted to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850. The postapproval report shall indicate the beginning and ending date of the period covered by the report and shall include the following information required by 21 CFR 814.84:

(1) Identification of changes described in 21 CFR 814.39(a) and changes required to be reported to FDA under 21 CFR 814.39(b).

(2) Bibliography and summary of the following information not previously submitted as part of the PMA and that is known to or reasonably should be known to the applicant:

(a) unpublished reports of data from any clinical investigations or nonclinical laboratory studies involving the device or related devices ("related" devices include devices which are the same or substantially similar to the applicant's device); and

(b) reports in the scientific literature concerning the device.

If, after reviewing the bibliography and summary, FDA concludes that agency review of one or more of the above reports is required, the applicant shall submit two copies of each identified report when so notified by FDA.

ADVERSE REACTION AND DEVICE DEFECT REPORTING. As provided by 21 CFR 814.82(a)(9), FDA has determined that in order to provide continued reasonable assurance of the safety and effectiveness of the device, the applicant shall submit 3 copies of a written report identified, as applicable, as an "Adverse Reaction Report" or "Device Defect Report" to the PMA Document Mail Center (HFZ-401), Center for Devices and Radiological Health, Food and Drug Administration, 9200 Corporate Blvd., Rockville, Maryland 20850 within 10 days after the applicant receives or has knowledge of information concerning:

(1) A mixup of the device or its labeling with another article.

(2) Any adverse reaction, side effect, injury, toxicity, or sensitivity reaction that is attributable to the device and

(a) has not been addressed by the device's labeling or

(b) has been addressed by the device's labeling, but is occurring with unexpected severity or frequency.

(3) Any significant chemical, physical or other change or deterioration in the device or any failure of the device to meet the specifications established in the approved PMA that could not cause or contribute to death or serious injury but are not correctable by adjustments or other maintenance procedures described in the approved labeling. The report shall include a discussion of the applicant's assessment of the change, deterioration or failure and any proposed or implemented corrective action by the applicant. When such events are correctable by adjustments or other maintenance procedures described in the approved labeling, all such events known to the applicant shall be included in the Annual Report described under "Postapproval Reports" above unless specified otherwise in the conditions of approval to this PMA. This postapproval report shall appropriately categorize these events and include the number of reported and otherwise known instances of each category during the reporting period. Additional information regarding the events discussed above shall be submitted by the applicant when determined by FDA to be necessary to provide continued reasonable assurance of the safety and effectiveness of the device for its intended use.

REPORTING UNDER THE MEDICAL DEVICE REPORTING (MDR) REGULATION. The Medical Device Reporting (MDR) Regulation became effective on December 13, 1984, and requires that all manufacturers and importers of medical devices, including in vitro diagnostic devices, report to FDA whenever they receive or otherwise became aware of information that reasonably suggests that one of its marketed devices

(1) may have caused or contributed to a death or serious injury or

(2) has malfunctioned and that the device or any other device marketed by the manufacturer or importer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

The same events subject to reporting under the MDR Regulation may also be subject to the above "Adverse Reaction and Device Defect Reporting" requirements in the "Conditions of Approval" for this PMA. FDA has determined that such duplicative reporting is unnecessary. Whenever an event involving a device is subject to reporting under both the MDR Regulation and the "Conditions of Approval" for this PMA, you shall submit the appropriate reports required by the MDR Regulation and identified with the PMA reference number to the following office:

Division of Surveillance Systems (HFZ-531)
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Room 240
Rockville, Maryland 20850
Telephone (301) 594-2735

Events included in periodic reports to the PMA that have also been reported under the MDR Regulation must be so identified in the periodic report to the PMA to prevent duplicative entry into FDA information systems.

Copies of the MDR Regulation and an FDA publication entitled, "An Overview of the Medical Device Reporting Regulation," are available by written request to the address below or by telephoning 1-800-638-2041.

Division of Small Manufacturers Assistance (HFZ-220)
Center for Devices and Radiological Health
Food and Drug Administration
5600 Fishers Lane
Rockville, Maryland 20857

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Generic Name: *In vitro* reagent system for the quantitative measurement of tacrolimus in human whole blood samples.

Trade Name: IMx Tacrolimus II Assay

Applicant's Name & Address: Abbott Laboratories
200 Abbott Park Road
Abbott Park, Illinois 60064-3537

Premarket Approval Application (PMA) Number: P970007

Date of Panel Recommendation: Pursuant to section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Clinical Chemistry and Toxicology Advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel for cyclosporin assay (P920031).

Date of Notice of Approval to the Applicant: August 26, 1997

II. Indication for Use

The IMx Tacrolimus II assay is an *in vitro* reagent system for the quantitative determination of tacrolimus and some metabolites in human whole blood as an aid in the management of liver allograft patients receiving tacrolimus therapy.

Background

Tacrolimus is an immunosuppresive drug discovered in 1984 by Fujisawa Pharmaceutical Co., Ltd. It has been shown to be effective for the treatment of rejection following transplantation.[1] The results of clinical trials with liver therapy[2] and kidney therapy.[3,4] have been published. The mode of action for tacrolimus is under active investigation. Tacrolimus binds a family of proteins termed FK506 (tacrolimus) binding proteins (FKBPs).[5,6] The formation of a larger pentameric complex comprised of FKBP, tacrolimus, calmodulin, and calcineurins A and B results in the inhibition of the phosphatase activity of calcineurin.[7] The action of transcription factors requiring

12

dephosphorylation for transport to the cell nucleus are inhibited leading to blockage of T-cell proliferation and function.

Tacrolimus may be administered intravenously or orally. Absorption from the gastrointestinal tract is variable and irregular.[5,6] Pharmacokinetic studies with tacrolimus have shown that there are large inter- and intra- individual differences in its kinetics in organ transplant patient.[9,10]

Pharmacokinetics studies have also indicated that whole blood rather than plasma may serve as the more appropriate medium to describe the pharmacokinetic characteristics of tacrolimus. Tacrolimus is bound to proteins, mainly albumins, and alpha- I - acid glycoprotein, and is highly bound to erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors such as hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration. In a U.S. study the ratio of whole blood concentration to plasma concentration ranged from 12 to 67 (mean 35).[11]

Tacrolimus is extensively metabolized in the liver and small intestine microsomes utilizing the hepatic cyctochrome P450 enzymes.[12] Nine different metabolites of tacrolimus have been identified; several of the metabolites have been found and tested in whole blood.[13-17] At the present time it is not clear whether the nephrotoxicity of tacrolimus is the result of parent drug, metabolites, or a combination of both.

The use of tacrolimus is associated with toxic side effects, primarily nephrotoxicity.[18-19] Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea.[20]

III. Device Description

The IMx Tacrolimus II assay is an *in vitro* reagent system for the quantitative determination of tacrolimus and some metabolites in human whole blood as an aid in the management of liver allograft patients receiving tacrolimus therapy.

The reagent pack contains 4 bottles with the following components:

<u>Imx Tacrolimus II Reagent Pack</u>

1. 1 Bottle (8.0 mL) Anti-Tacrolimus (Mouse, Monoclonal) Antibody Coated Microparticles in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.0015 percent solids. Preservatives: Sodium azide and antimicrobial agents.

2. 1 Bottle (9.5 mL) Tacrolimus Alkaline Phosphate Conjugate in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 1µg/mL. Preservatives: Sodium azide and antimicrobial agents.

3. 1 Bottle (10 mL) 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: Sodium azide.

4. 1 Bottle (10.3 mL) Wash Solution. Preservative: Sodium azide.

The IMx Tacrolimus II assay employs Microparticle Enzyme Immunoassay (MIEIA) technology with front surface fluorescence detection. The presence of erythrocytes in the sample matrix requires a pretreatment step to extract tacrolimus and remove hemoglobin. In the IMx Tacrolimus II assay protocol, 150 uL aliquots of calibrators, controls or patient specimen (whole blood) are mixed with 150 uL of precipitation reagent.

After vortexing and centrifugation, the supernatants are poured into the sample compartment of the IMx reaction cell. The loaded reaction cells are positioned on the IMx carousel which is placed into the IMx Analyzer together with the IMx Tacrolimus II reagent pack. The IMx Tacrolimus II reagent pack contains a vial of microparticles, a vial of conjugate, a vial of substrate and a vial of wash solution. The remainder of the assay is automated. The IMx Analyzer probe/electrode assembly delivers an aliquot of the sample, microparticles, and conjugate to the incubation well of the reaction cell. The tacrolimus from the sample and the conjugate competitively bind to the microparticles forming "antibody-antigen" and "antibody-antigen~-alkaline phosphatase" complexes. An aliquot of the reaction mixture containing the "antibody-antigen" and "antibody-antigen-alkaline phosphatase" complexes bound to the microparticles is transferred to the glass fiber matrix. The microparticles bind irreversibly to the glass fiber matrix. The matrix is washed to remove unbound materials. The substrate is added to the matrix and the fluorescent product is measured by the MEIA optical assembly, a front surface fluorometer that uses a mercury arc lamp as its light source. Emitted light is directed through an excitation filter which selects light with a nominal wavelength of 365 nm. This light is then focused onto the surface of the reaction cell matrix.

The fluorescent product absorbs the excitation light and rises to its excited state. Upon returning to its ground state, the fluorescent product emits light at 448 nm. The emitted light passes through a dual filtering system that selects the appropriate wavelength of light which is then focused on the photomultiplier tube. The resulting light intensity is measured

and used to calculate the concentration of analyte (tacrolimus and some metabolites) in the test sample. The results are printed on the data tape.

CONTRAINDICATIONS:

There are no known contraindications for the IMx Tacrolimus II assay.

WARNINGS AND PRECAUTIONS

Warnings and Precautions for use of the device are stated in the attached product labeling. (Attachment A)

IV. Alternate Practices and Procedures

Alternate methods to measure tacrolimus have been described in literature including a radioreceptor assay, high-performance liquid chromatography-mass spectrometry (HPLC-MS), high-performance liquid chromatography (HPLC) and ELISA methodologies.

V. Marketing History

The Imx Tacrolimus II Assay has not been marketed in the United States or any other country.

VI. Adverse Effects of the Device on Health

A falsely elevated tacrolimus whole blood concentration could result in the physicain lowering the tacrolimus dosage. As a result of the lowered dose of tacrolimus the immunosupressive ability of tacrolimus may not be effective enough to impede a cellular response, causing an adverse event of rejection. A falsely lowered tacrolimus whole blood concentration could result in the physician raising the tacrolimus dosage. As a result of the increased dose of tacrolimus, the patient may be subjected to an adverse event of toxicity.

VII. Summary of Studies

A. Summary of Non-Clinical Studies

The performance characteristics of the IMx Tacrolimus II assay were evaluated in-house and at five external clinical sites. The five external clinical sites performed testing for precision, sensitivity, two-week calibration curve stability, and specimen correlation. These tests were performed according to the IMx Tacrolimus II Clinical Protocol. Additional studies were performed to further define the assay performance characteristics, e.g. functional sensitivity, additional interferences, and metabolites.

16

Precision

The IMx Tacrolimus II precision studies from the five clinical sites and in-house testing for the Low Control (5 ng/mL), Medium Control (11 ng/mL), and High Control (22 ng/mL) followed the NCCLS Guideline "Evaluation of Precision Performance of Clinical Chemistry Devices" (EP5-T2). These control products are prepared with processed human whole blood nonreactive for HB_sAG, Anti-HCV, and Anti-HIV-1/HIV-2. The data for total percent coefficient of variation (CV) and standard deviation (SD) are pooled estimates of precision from three clinical lots tested on ten instruments are in Table 1.

Table 1

Control	n	Mean	Total SD	Total %CV
Low (5ng/mL)	800	5.02	0.60	12.0
Medium (11 ng/mL)	800	10.89	0.86	7.9
High (22 ng/mL)	800	21.42	1.41	6.6

In-house precision studies for tacrolimus whole blood concentrations at 1.5, 2, and 3 ng/lL followed the NCCLS Guideline "Evaluation of Precision Performance of Clinical Chemistry Devices" (EP5-T2). The data for total percent CV and SD are pooled estimates of precision from four lots tested on six instruments are in Table 2.

Table 2

Sample	n	Mean	Total SD	Total %CV
1 (1.5 ng/mL)*	480	1.84	0.41	22.2
2 (2 ng/mL)	480	2.36	0.39	16.3
3 (3 ng/mL)	480	3.36	0.39	11.5

* 1. 5 ng/mL is the analytical sensitivity of the IMx Tacrolimus II assay.

Sensitivity

The functional and analytical sensitivity for the IMx Tacrolimus II assay were determined at the five clinical sites. The analytical sensitivity, determined from the Mode I Calibrator rates, was analyzed using a non-parametric statistical method. At the lower 2.5 percent quantile, the sensitivity or minimum detection level was 1.5 ng/mL.

The functional sensitivity was determined by serially diluting 25

individual tacrolimus whole blood specimens with Mode I calibrator (0 ng/mL), and determining where the observed value fell outside the +/- 20 percent tolerance limits. The functional sensitivity ranged from the analytical sensitivity to 4.1 ng/mL.

Accuracy by Correlation

Accuracy by correlation testing was performed in-house for the IMx Tacrolimus II assay versus HPLC/MS/MS. A total of 105 tacrolimus whole blood clinical specimens was analyzed. The correlation analysis produced a slope of 0.94, intercept of 0.63 and a R of 0.987.

Recovery

Recovery for the IMx Tacrolimus II assay was determined in-house by testing tacrolimus-free whole blood specimens spiked with known amounts of tacrolimus. The recovery of tacrolimus was determined using the observed values as compared to the theoretical values. The theoretical concentrations tested were 2.0, 3.0, 6.0, 12.0, 20.0 and 25.0 ng/mL. The recovery ranged from 87.5 percent to 115.0 percent (mean = 96.2 percent).

Interfering Substances

Interference testing was performed in-house for cholesterol, triglycerides, uric acid, heparin, bilirubin, protein, and hematocrit.

Specimen and sample types were defined as follows:
Specimen = clinical specimen (unprocessed whole blood)
Sample = Calibrator and/or Control (processed whole blood matrix)

Cholesterol

Specimens containing endogenous cholesterol ranging from 208 to 366 mg/dL were tested at tacrolimus concentrations of 1.5 and 11 ng/mL resulted in less than 15 percent error in detecting tacrolimus.
Triglycerides

Samples at tacrolimus concentrations of 1.5, 5, 11, and 22 ng/mL that were prepared using the whole blood matrix and spiked with 400 and 800 mg/dL triglycerides, resulted in less than 10 percent error in detecting tacrolimus. Specimens containing elevated endogenous triglyceride levels ranging from 268 to 988 mg/dL were spiked with known concentrations of tacrolimus at 1.5 and 11 ng/mL resulted in less than 10 percent error in detecting tacrolimus at 11 ng/mL.

Uric Acid

Samples at tacrolimus concentrations of 1. 5, 5, 11, and 22 ng/mL were prepared using the whole blood matrix and spiked with 20 and 40 mg/dL uric acid. Uric acid concentrations up to 40 mg/dL resulted in less than 10 percent error in detecting tacrolimus.

Heparin

Specimens were drawn into both EDTA and heparin tubes. These specimens were spiked with tacrolimus to a concentration of 1, 5, 5, 11, and 22 ng/mL. The percent interference was calculated by comparing the heparin tube (test) to the corresponding EDTA tube (control). The percent interference with the IMx Tacrolimus II assay for heparin tubes was less than 15 percent error in detecting tacrolimus at concentrations >1.5 ng/mL. EDTA anti-coagulated tubes will be used with the IMx Tacrolimus II assay.

Bilirubin

Samples at tacrolimus concentrations of 5, 11, and 22 ng/mL were prepared using the whole blood matrix and spiked with 20, 30, and 40 mg/dL of bilirubin. Bilirubin concentrations up to 40 mg/dL resulted in less than 10 percent error in detecting tacrolimus.

Protein

Protein interference was performed using a control whole blood specimen at 35 percent hematocrit and 17 g/dL protein. Aliquots of the control specimen were spiked with protein (human serum albumin) or diluted with saline to prepare specimens at 15, 21, and 25 g/dL protein. Each specimen was spiked with tacrolimus to concentrations of 5, 11, and 22 ng/mL. Protein concentrations up to 25 g/dL resulted in less than 11 percent error in detecting tacrolimus.

Hematocrit

A specimen was prepared to obtain a hematocrit value of 45 percent. This specimen was diluted with saline to obtain specimens at 25 and 35 percent hematocrit. Each specimen was spiked with tacrolimus to concentrations of 5, 11, and 22 ng/mL. Hematocrit concentrations up to 45 percent resulted in less than 10 percent error in detecting tacrolimus.

Cross-Reactivity

Metabolite Cross-reactivity:

The cross-reactivity of seven tacrolimus metabolites was evaluated. The metabolites were provided by Fujisawa Pharmaceutical Co., Ltd., Japan, and are not commercially available in a purified form for routine product release testing. The metabolites were tested in the absence of tacrolimus. Cross-reactivity occurred with three metabolites (M-II, M-III and M-V) as summarized in the table 3:

Table 3

Metabolite	Spiked Concentration	Mean Measured Concentration	% Cross-Reactivity *
M-I	10 ng/mL	< 1.5 ng/mL	N/A**
M-II	10 ng/mL	5.4 ng/mL	54.0%
M-III	10 ng/mL	6.7 ng/mL	67.0%
M-IV	10 ng/mL	< 1.5 ng/mL	N/A
M-V	10 ng/mL	6.2 ng/mL	62.0%
M-VI	10 ng/mL	< 1.5 ng/mL	N/A
M-VII	10 ng/mL	< 1.5 ng/mL	N/A
M-VIII	10 ng/mL	< 1.5 ng/mL	N/A

* Cross-reactivity (tacrolimus measured/metabolite spiked).
**Cross-reactivity could not be determined for concentrations less than assay sensitivity.

Tacrolimus metabolites M-II, M-III, and M-V were tested at 10 ng/mL in the prescences of 11 ng/mL tacrolimus. Metabolites M-II showed a doubling of the Tacrolimus concentration while M-III and M-V showed a 20-40 percent increase in the Tacrolimus concentration. All additional compounds tested either in the presence or absence of tacrolimus yielded concentrations less than the sensitivity of the assay. See Table 4.

Table 4

The following compounds were tested at the stated concentrations in the absence of tacrolimus and yielded concentrations less than the analytical sensitivity of the assay 1.5 ng/mL:

Test Compound	Test Concentration
N-Acetylprocainamide	100 ug/mL
Acyclovir	1000 ug/mL
Amikacin	100 ug/mL
Amphotericin B	100 ug/mL
Azathioprine	100 ug/mL
Carbamazepine	100 ug/mL
Cephalosporine	100 ug/mL
Chloramphenicol	100 ug/mL
Cimetidine	100 ug/mL
Cyclosporine	1000 ng/mL
Digitoxin	100 ng/mL
Digoxin	10 ng/mL
Disopyramide	5 ug/mL
Erythromycin	100 ug/mL
Furosemide	100 ug/mL
Ganciclovir	1000 ug/mL
Kanamycin	100 ug/mL
Lidocaine	100 ug/mL
Mycophenolic Acid	200 ug/mL
Penicillin	100 ug/mL
Phenytoin	100 ug/mL
Prazosin	25 ug/mL
Prednisolone	100 ug/mL
Prednisone	100 ug/mL
Primidone	100 ug/mL
Procainamide	100 ug/mL
Quinidine	100 ug/mL
Rifampin	100 ug/mL
Spectinomycin	100 ug/mL
Valproic Acid	1000 ug/mL
Vancomycin	100 ug/mL
Verapamil	10 ug/mL

Clinical Specimen Duplicate Analysis

Results of the duplicate testing analysis demonstrated that, with 95 percent confidence, there was no statistical difference between the first replicate and the second replicate. Therefore, it is not necessary to assay specimens in duplicate.

Calibration Curve Stability

The data collected from the five clinical sites supported the two-week calibration curve stability.

Clinical Specimen Stability

Tacrolimus whole blood clinical specimen stability was evaluated at three storage conditions. The clinical specimen stability testing demonstrated that tacrolimus clinical specimens can be stored at 2-8°C for 28 days, 37°C for 3 days followed by 2-8°C for 11 days and -10°C for 56 days prior to being evaluated using the IMx Tacrolimus II assay.

Product Transport and Storage

Three lots of IMx Tacrolimus II assay reagent, calibrators and controls were subjected to various temperature conditions to determine the best transport, and storage temperatures for product stability. The stability results supported the product stability for intended storage (2- 8 °C) and shipping temperatures (reagent on ice and calibrator and control on dry ice) at nine months for reagents, calibrators, and controls.

B. Summary of Clinical Data

A total of 8 sites participated in the IMx Tacrolimus II assay clinical study that began in December, 1995.

A total of 102 liver transplant patients was included in the clinical data analysis. Each patient was evaluated for a minimum of 80 days post-transplant. A total of 2604 tacrolimus concentrations from the 102 patients was measured using the IMx Tacrolimus II assay.

Of the 102 liver transplant patients, 96 were adult (> 12 years of age), six were pediatric, (≤ 12 years of age), 55 (53.9 percent) were female, and 47 (46.1 percent) were male. Thirty-six (35.3 percent) of the 102 patients received CellCept (Roche Laboratories) as part of their immunosuppression therapy.

The pharmacokinetics of tacrolimus were the most variable during

21

the inital three weeks post-transplant compared to the remaining 11 weeks of the study. The variability in the individual patient pharmacokinetic profile was associated with a higher pooled within patient SD for tacrolimus whole blood concentrations and a greater number of adverse events seen during the initial three weeks post-transplant as compared to the last 11 weeks. An analysis of the clinical data by week for all patients resulted in the data for mean total daily tacrolimus dose and mean tacrolimus whole blood concentrations shown in Table 5.

Table 5

Mean Total Tacrolimus Daily Dose
and IMx Tacrolimus II Whole Blood Concentrations
All Patients by Week

Week Post-transplant	Total Daily Dose mgs / SD	Tacrolimus Concentration ng/mL / SD
1	7.33 / 4.82	11.43 / 5.14
2	8.27 / 5.15	11.50 / 4.14
3	8.01 / 4.76	10.64 / 4.50
4	8.37 / 4.89	10.14 / 4.57
5	8.74 / 4.90	10.23 / 5.45
6	8.29 / 4.49	9.68 / 4.12
7	8.41 / 4.61	10.37 / 4.86
8	8.64 / 4.57	10.33 / 4.94
9	8.83 / 4.69	9.85 / 4.93
10	8.75 / 4.23	9.76 / 3.90
11	8.75 / 4.57	9.49 / 3.40
12	7.94 / 4.46	9.23 / 4.23
13	8.00 / 4.72	9.58 / 4.07
14	7.44 / 4.16	9.24 / 3.82

The data for adverse events of nephrotoxicity or rejection by week followed by the percent from the total number of adverse events are listed in Table 6.

Table 6

Adverse Events
Nephrotoxicity and Rejection
All Patients by Week

Week	Nephrotoxicity	Rejection	Total
1	9 (19.6%)	16 (30.2%)	25 (25.3%)
2	3 (6.5%)	13 (24.5%)	16 (16.2%)
3	5 (10.9%)	5 (9.4%)	10 (10.1%)
4	1 (2.2%)	5 (9.4%)	6 (6.1%)
5	3 (6.5%)	3 (5.7%)	6 (6.1%)
6	3 (6.5%)	5 (9.4%)	8 (8.1%)
7	3 (6.5%)	1 (1.9%)	4 (4.0%)
8	3 (6.5%)	2 (3.8%)	5 (5.1%)
9	4 (8.7%)	0 (0.0%)	4 (4.0%)
10	3 (6.5%)	0 (0.0%)	3 (3.0%)
11	3 (6.5%)	0 (0.0%)	3 (3.0%)
12	2 (4.3%)	0 (0.0%)	2 (2.0%)
13	1 (2.2%)	0 (0.0%)	1 (1.0%)
14	3 (6.5%)	3 (5.7%)	6 (6.1%)
Total	46 (100%)	53 (100%)	99 (100%)

The mean values for the total daily tacrolimus dose and the tacrolimus whole blood concentrations across all patients in the study remained relatively constant from the time of transplant through the fourteenth week post-transplant. However, there was a decrease in the number of adverse events over this 14 week period post-transplant.

The relationship between the dosing of tacrolimus, the whole blood tacrolimus concentrations, and adverse events could not be clearly defined.

VIII. Conclusion Drawn From The Studies

The data presented in the Non-clinical Performance Testing and the Clinical Laboratory Studies demonstrated that the IMx Tacrolimus II assay was safe and effective as an aid in measuring whole blood tacrolimus concentrations in patients receiving tacrolimus therapy.

The precision testing has demonstrated that the total CVs ranged from 6.6 percent for high tacrolimus concentrations approximately at 22 ng/mL to 16.3 percent for low tacrolimus concentration approximately at 2 ng/mL.

The accuracy by correlation data demonstrated that IMx Tacrolimus II assay was comparable to the HPLC/MS/MS in measuring tacrolimus whole blood concentrations in patients receiving tacrolimus therapy.

The analytical sensitivity at the lower 2.5 percent quantile using non-parametric statistical method was 1.5 ng/mL. The functional sensitivity was 4.1 ng/mL.

The interference testing demonstrated that there was no significant interfering substances with the IMx Tacrolimus II assay. Cholesterol, triglycerides, uric acid, heparin, bilirubin, protein, and hematocrit were tested and the assay demonstrated less than 15 percent error in detecting tacrolimus concentrations above the analytical sensitivity of the assay.

The cross-reactivity testing demonstrated that the IMx Tacrolimus II assay had some cross-reactivity with three metabolites of tacrolimus M-II, M-III and M-V. The clinical significance or physiological levels of these metabolites have not been clearly defined.

The calibration curve stability testing demonstrated that the calibration curve was stable for a minimum of two weeks.

The clinical specimen stability testing demonstrated that the clinical specimens could be stored at 2-8°C for 28 days, 37°C for 3 days followed by 2-8°C for 11 days, and -10°C for 56 days prior to being evaluated using the IMx Tacrolimus II assay.

The IMx Tacrolimus II Reagent, Calibrator and Control stability testing demonstrated a product expiration dating of nine months.

The clinical data demonstrated that there was not a clear relationship between the tacrolimus dosing, whole blood concentrations of tacrolimus, and adverse events of nephrotoxicity or rejection. This was most evident during the initial three weeks post-transplant when the individual patient pharmacokinetic profile of tacrolimus was the most variable. This variability in the pharmacokinetics of tacrolimus was associated with an increased number of adverse events that occurred during this initial three week period post-transplant, although the mean total daily tacrolimus dose and mean whole blood concentrations remained relatively constant throughout the study.

The results of this study demonstrated that the complexity of the clinical state and individual differences in sensitivity to the immunosuppressive effects of tacrolimus will cause different requirements for optimal whole blood levels of tacrolimus. Each patient should be thoroughly evaluated clinically before treatment adjustments are made. The physician should establish individual patient ranges based on these clinical evaluations. Individual whole blood tacrolimus values cannot be used as the sole indicator for making changes in the treatment regimen. These data supported the intended use for the quantitative determination of tacrolimus and some metabolites in human whole blood as an aid in management of liver allograft patients receiving tacrolimus therapy.

In addition, tacrolimus blood concentrations have been observed by this IMx method in the drug clinical studies that let to its approval.

IX. Panel Recommendation

Pursuant to section 515(c)(2) of the act as amended by the safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Clinical Chemistry and Toxicology Devices advisory Panel meeting because the information in the PMA substantially duplicates information previously reviewed by this panel.

X. CDRH Action on the Application

CDRH issued an approval order for the applicant's PMA for the IMx

Tacrolimus II assay on August 26, 1997.

The applicant's manufacturing and control facilities were inspected on and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMPs). The shelf-life of IMx Tacrolimus II Reagent Pack at 2 to 8°C is nine months.

XI. Approval Specifications

Directions for use: See labeling

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order.

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28

TRANSPLANT DIAGNOSTICS

IM® SYSTEM

Tacrolimus II

Note Changes and Important Information Highlighted

U.S. Patent 5,338,684

66-9474/R2

To run the IMx Tacrolimus II Assay you must first edit two assay parameters. The MODE 1 (parameter #112.5) must be edited to run in duplicate. The MAX CHECK 1 (parameter #112.42) must be edited to 0.890. To edit assay parameters refer to the **INSTRUMENT PROCEDURE** section, IMx TACROLIMUS II ASSAY PARAMETERS in this package insert.

CAUTION: United States Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by or on the order of a physician.

WARNING: Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which utilize mouse monoclonal antibodies. These specimens should not be assayed with the IMx Tacrolimus II assay. Refer to the **LIMITATION OF THE PROCEDURE** section of this package insert.

NOTE: This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from this package insert.

ABBOTT LABORATORIES
Diagnostics Division
Abbott Park, IL 60064

July, 1997

Printed in U.S.A.
List No. 3C10

35

NAME

Tacrolimus II

INTENDED USE

The IMx® Tacrolimus II assay is an *in vitro* reagent system for the quantitative determination of tacrolimus and some metabolites in human whole blood as an aid in the management of liver allograft patients receiving tacrolimus therapy.[1]

SUMMARY AND EXPLANATION OF THE TEST

Tacrolimus is an immunosuppressive drug discovered in 1984 by the Fujisawa Pharmaceutical Co., Ltd. It has been shown to be effective for the treatment of rejection following transplantation. The results of clinical trials with liver[2] and kidney[3,4] have been published. Clinical studies are continuing for a variety of indications.

The mode of action for tacrolimus is under active investigation. Tacrolimus binds a family of proteins termed FK506 (tacrolimus) binding proteins (FKBP's).[5,6] The formation of a larger pentameric complex comprised of FKBP, tacrolimus, calmodulin and calcineurins A and B results in the inhibition of the phosphatase activity of calcineurin.[7] The action of transcription factors requiring dephosphorylation for transport to the cell nucleus are thus inhibited leading to blockage of T-cell proliferation and function.

Tacrolimus may be administered IV or orally. Absorption from the gastrointestinal tract is variable and irregular.[8] Pharmacokinetic studies with tacrolimus have shown that there are large inter- and intra individual differences in its kinetics in organ transplant patients.[9,10]

Pharmacokinetic studies have also indicated that whole blood rather than plasma may serve as the more appropriate medium to describe the pharmacokinetic characteristics of tacrolimus. Tacrolimus is bound to proteins, mainly albumins, and alpha-1-acid glycoprotein, and is highly bound to erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors such as hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration. In a U.S. study, the ratio of whole blood concentration to plasma concentration ranged from 12 to 67 (mean 35).[11]

Tacrolimus is extensively metabolized in the liver and small intestine microsomes utilizing the hepatic cytochrome P-450 enzymes.[12] Nine different metabolites of tacrolimus have been identified; several of the metabolites have been found and tested in whole blood.[13-17] At the present time it is not clear whether the nephrotoxicity of tacrolimus is the result of parent drug, metabolites, or a combination of both.

The use of tacrolimus is associated with serious toxic side effects, primarily nephrotoxicity.[18,19] Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea.[20]

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The IMx Tacrolimus II assay is based on the Microparticle Enzyme Immunoassay (MEIA) technology. Prior to initiation of the automated IMx sequence, a manual pretreatment step is performed in which the whole blood sample is extracted with a precipitation reagent and centrifuged. The supernatant is decanted into the sample well and the IMx Tacrolimus II reagents and sample are added to the reaction cell in the following sequence:

- The probe/electrode assembly delivers the sample, Anti-Tacrolimus (Mouse, Monoclonal) Antibody Coated Microparticles, and Tacrolimus-Alkaline Phosphatase Conjugate to the incubation well of the reaction cell.
- The tacrolimus and conjugate competitively bind to the Anti-Tacrolimus Microparticles forming "antibody-antigen" and "antibody-antigen-alkaline phosphatase" complexes.
- An aliquot of the reaction mixture containing the "antibody-antigen" and "antibody-antigen-alkaline phosphatase" complexes bound to the microparticles is transferred to the glass fiber matrix. The microparticles bind irreversibly to the glass fiber matrix.
- The matrix is washed to remove unbound materials.
- The substrate, 4-Methylumbelliferyl Phosphate, is added to the matrix and the fluorescent product is measured by the MEIA optical assembly.

For further information refer to your IMx System Operation Manual, Section 3.

REAGENTS

REAGENT PACK

IMx Tacrolimus II Reagent Pack, 100 tests (No. 3C10-20)*

1. 1 Bottle (8.0 mL) Anti-Tacrolimus (Mouse, Monoclonal) Antibody Coated Microparticles in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.0015% solids. Preservatives: Sodium Azide and Antimicrobial Agents.
2. 1 Bottle (9.5 mL) Tacrolimus Alkaline Phosphatase Conjugate in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 1 µg/mL. Preservatives: Sodium Azide and Antimicrobial Agents.
3. 1 Bottle (10 mL) 4-Methylumbelliferyl Phosphate, 1.2 mM, in AMP buffer. Preservative: Sodium Azide.
4. 1 Bottle (10.3 mL) Wash Solution. Preservative: Sodium Azide.

*No. 3C10-66 includes an IMx Tacrolimus II Reagent Pack (100 tests), IMx Tacrolimus II Whole Blood Precipitation Reagent (100 tests), MEIA Reaction Cells (100 each) and centrifuge tubes (100 each).

PRECIPITATION REAGENT

IMx Tacrolimus II Whole Blood Precipitation Reagent (No. 3C10-55)

1 Bottle (18.8 mL) of IMx Tacrolimus II Whole Blood Precipitation Reagent. Zinc sulfate solution in methanol and ethylene glycol.

MODE 1 CALIBRATOR

IMx Tacrolimus II MODE 1 Calibrator (No. 3C10-40)

3 Bottles (4.5 mL each) of IMx Tacrolimus II MODE 1 Calibrator are prepared with processed human whole blood nonreactive for HBsAg, anti-HCV, and anti-HIV-1/HIV-2. Concentration: 0 ng/mL tacrolimus. Preservatives: Sodium Azide and Antimicrobial Agents.

NOTE: The IMx Tacrolimus II MODE 1 Calibrator (No. 3C10-40) must be ordered separately. It will not automatically be shipped with the IMx Tacrolimus II Reagent Pack.

CALIBRATORS

IMx® Tacrolimus II Calibrators (No. 3C10-01)

6 Bottles (4.5 mL each) of IMx Tacrolimus II Calibrators are prepared with processed human whole blood nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2. IMx Tacrolimus II Calibrators contain the following concentrations of tacrolimus:

Bottle	Tacrolimus Concentration (ng/mL)
A	0
B	3
C	6
D	12
E	20
F	30

Preservatives: Sodium Azide and Antimicrobial Agents.

CONTROLS

IMx Tacrolimus II Controls (No. 3C10-10)

3 Bottles (9.0 mL each) of IMx Tacrolimus II Controls are prepared with processed human whole blood nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2. IMx Tacrolimus II Controls contain the following concentration ranges:

Bottle	Tacrolimus Concentration (ng/mL)	Range (ng/mL)
L	5	3.0 - 7.0
M	11	7.7 - 14.3
H	22	15.4 - 28.6

Preservatives: Sodium Azide and Antimicrobial Agents.

OTHER REAGENTS

IMx Probe Cleaning Solution (No. 1A71-02)

1 Bottle (110 mL) IMx Probe Cleaning Solution containing 2% Tetraethylammoniumhydroxide (TEAH)

IMx MEIA #2 Diluent Buffer (No. 8374-04)

4 Bottles (1000 mL each) IMx MEIA #2 Line Diluent Buffer containing 0.3M Sodium Chloride in TRIS Buffer. Preservatives: Sodium Azide and Antimicrobial Agents.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

CAUTION: This product contains human sourced and/or potentially infectious components. For a specific listing, refer to the **REAGENTS** section of this package insert. Components sourced from human blood have been tested and found to be nonreactive for antibodies to HIV-1/HIV-2 and HCV and nonreactive for HBsAg. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[21] Biosafety Level 2[22] or other appropriate biosafety practices[23,24] should be used for materials that contain or are suspected of containing infectious agents.

Some components of this product contain Sodium Azide. For a specific listing, refer to the **REAGENTS** section of this package insert. The components containing Sodium Azide are classified per applicable European Economic Community (EEC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases:

R22 Harmful if swallowed.
R32 Contact with acids liberates very toxic gas.
S2 Keep out of the reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
S36 Wear suitable protective clothing.
S46 If swallowed, seek medical advice immediately and show this container or label.

The IMx Tacrolimus II Wash Solution contains Thiourea and Sodium Azide and is classified per applicable European Economic Community (EEC) Directives as: Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

R22 Harmful if swallowed.
R32 Contact with acids liberates very toxic gas.
R40 Possible risks of irreversible effects.
S2 Keep out of the reach of children.
S13 Keep away from food, drink and animal feedingstuffs.
S36/37 Wear suitable protective clothing and gloves.
S46 If swallowed, seek medical advice immediately and show this container or label.

The IMx Tacrolimus II Whole Blood Precipitation Reagent (No. 3C10-55) contains Methanol and Ethylene Glycol and is classified per applicable European Economic Community (EEC) Directives as: Toxic (T) Flammable (F). The following are the appropriate Risk (R) and Safety (S) phrases.

R10 Flammable.
R23/25 Toxic by inhalation and if swallowed.
S1/2 Keep locked up and out of the reach of children.
S7 Keep container tightly closed.
S16 Keep away from sources of ignition. NO SMOKING.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

The IMx Probe Cleaning Solution (No. 1A71-02) contains 2% TEAH and may cause mild eye irritation. If this solution comes in contact with the eyes, flush immediately with water.

The safety and handling precautions and limitations for the reagent pack, calibrators, controls and patient samples are described in your IMx System Operation Manual, Section 8.

The IMx Tacrolimus II assay results may be impacted when reaction cells from different lots are used within a carousel. Use only one lot of reaction cells within an IMx Tacrolimus II assay run. Store reaction cells in a manner that assures lot traceability.

SHIPPING AND STORAGE

NOTES: The IMx Tacrolimus II Reagents are shipped with cold packs. The IMx Tacrolimus II MODE 1 Calibrator, Calibrators and Controls are shipped on dry ice and should be stored at 2-8°C after receipt. The MODE 1 Calibrator, Calibrators and Controls must be completely thawed and mixed thoroughly before beginning a run.

The storage condition for the IMx Tacrolimus II Reagent Pack, MODE 1 Calibrator, Calibrators and Controls is 2-8°C. The IMx Tacrolimus II Whole Blood Precipitation Reagent should be stored at 15-30°C. The reagent pack, calibrators and controls can be used **immediately** after removing them from the refrigerator.

The IMx Tacrolimus II Reagents are stable until the expiration date when stored and handled as directed. Do not use past the expiration date.

Store reaction cells in the box to assure lot traceability.

INSTRUMENT PROCEDURE

The following instrument software is required to perform the assay:

- IMx System Software Module Version 6.0 or higher
- IMx TDM/Transplant Assay Module Version 3.0 or higher

ASSAY ACTIVATION

Prior to running the IMx® Tacrolimus II assay, activation of the assay is required. Refer to the Index in the IMx System Operation Manual to locate the Assay Activation procedure. Assay Activation for the IMx Tacrolimus II is only required prior to the first Tacrolimus II assay run with the IMx TDM/Transplant Assay Module Version 3.0, or after a FAC_SET ALL or FAC_SET ASSAY has been performed.

IMx TACROLIMUS II ASSAY PARAMETERS

The following IMx Tacrolimus II assay parameters have been factory set. These parameters can be printed, displayed and edited according to the procedure in your IMx System Operation Manual, Section 6. Ensure that the assay parameters for the IMx Tacrolimus II assay in the assay module match these parameters or edit accordingly.

NOTE: TO RUN THE IMx TACROLIMUS II ASSAY YOU MUST FIRST EDIT TWO ASSAY PARAMETERS, 112.5 AND 112.42.

To run the MODE 1 in duplicate, edit assay parameter 112.5 (M1 CAL REP) from 1 to 2 as follows:

Press [ASSAY]
Enter [112.5]
Press [DISPLAY]
Enter [2]
Press [STORE]
Press [EXIT] 2 times

To enter the correct MAX CHECK 1 parameter, edit assay parameter 112.42 from 0.870 to 0.890 as follows:

Press [ASSAY]
Enter [112.42]
Press [DISPLAY]
Enter [0.890]
Press [STORE]
Press [EXIT] 2 times

The assay parameters that cannot be edited are noted with an asterisk (*).

Assay #112 IMx Tacrolimus II

1.	DECIMAL	1
2.	RUN DEFAULT	1
3.	SAMPLE REP	1
4.	CAL REP	2
5.	**M1 CAL REP**	**2**
* 6.	CONC A	0.000
* 7.	CONC B	3.000
* 8.	CONC C	6.000
* 9.	CONC D	12.000
* 10.	CONC E	20.000
* 11.	CONC F	30.000
12.	RESULT UNIT	1
13.	LOW LIMIT	-9999.000
14.	HIGH LIMIT	9999.000
15.	C1 LOT ID	00000000
* 16.	C1 DATE	11/11/11
* 17.	C1 TIME	0:00:00
* 18.	C2 LOT ID	00000000
* 19.	C2 DATE	11/11/11
* 20.	C2 TIME	0:00:00
* 27.	LOW RANGE	0.000
* 28.	HIGH RANGE	30.000
* 29.	MIN TRACER	-9999.00
* 30.	MAX BKG	9999.0
* 31.	MIN RATE	50.0
* 32.	MAX NRMSE	0.100
33.	MIN CORR	0.950
34.	MAX INTRCPT	15000.0
35.	MAX DEV	15.00
* 36.	MIN POL	-9999.00
37.	MIN READ	200.0
38.	MAX READ	1000.0
39.	MIN SPAN F-A	-9999.000
40.	MAX SPAN F-A	9999.000
41.	MIN CHECK 1	0.590
42.	**MAX CHECK 1**	**0.890**
43.	MIN CHECK 2	-9999.000
44.	MAX CHECK 2	9999.000
45.	MIN CHECK 3	0.320
46.	MAX CHECK 3	0.550
47.	MIN CHECK 4	-9999.000
48.	MAX CHECK 4	9999.000
49.	MIN CHECK 5	0.160
50.	MAX CHECK 5	0.320
* 52.	DIL FACT 1	1.000
* 53.	DIL FACT 2	1.000
* 54.	DIL FACT 3	1.000
* 55.	DIL FACT 4	1.000
* 56.	DIL FACT 5	1.000
57.	DIL DEFAULT	0
* 58.	LOW GRAY	-9999.000
* 59.	HIGH GRAY	9999.000
60.	PRINT OPTION	0
* 61.	CUTOFF	0.000
* 87.	MX MODE1 DEV	0.350

NOTE: RESULT UNIT, assay parameter 112.12, can only be edited to "1" (ng/mL) and PRINT OPTION, assay parameter 112.60, can only be edited to "0" or "1". Editing to another number will result in the displayed code "103 BAD VALUE IN ASSAY FILE 12 or 60", respectively, when the assay run is initiated. For further information on Changing Concentration Units and Print Options, refer to your IMx System Operation Manual, Section 5.

Refer to your IMx System Operation Manual for a detailed discussion of instrument procedures.

SPECIMEN COLLECTION AND STORAGE

- Only whole blood specimens (EDTA) may be used with the IMx Tacrolimus II assay.
- Specimens collected in EDTA tubes may be stored for up to 14 days at 2-8°C prior to being tested. (Recovery range 90.5-114.7%, stored in polypropylene tubes, tacrolimus range 4.5-19.1 ng/mL)
- Specimens which are not tested within 14 days must be stored frozen (-10°C or colder). Specimens must be mixed thoroughly after thawing to ensure consistency of the results. Avoid repeated freezing and thawing. (Recovery range 83.6-101.7%, stored in polypropylene tubes, tacrolimus range 13.7-23.5 ng/mL)
- No significant differences or trending were observed when tacrolimus specimens were subjected to 3 days at 37°C followed by 11 days at 2-8°C storage. (Recovery range 73.4-112.5%, stored in polypropylene tubes, tacrolimus range 5.6-23.8 ng/mL)
- The Consensus Document reports that drug recovery in patient whole blood has been reported ≥90% at 6 months, but loss of 46% occurs by 9 months[1].

77

SAMPLE VOLUME

150 µL of specimen is the minimum volume required for the Manual Pretreatment Step.

150 µL of pretreated specimen is the minimum volume required to perform the assay.

IMx® TACROLIMUS II PROCEDURE

Materials Required but Not Provided

- X-SYSTEMS® Centrifuge (No. 9527-25)
- Vortex Mixer
- Precision pipettor with disposable tips to accurately dispense 150 µL of whole blood
- X-SYSTEMS Precision Dispenser (List No. 9528-02) for dispensing 150 µL of IMx Tacrolimus II Whole Blood Precipitation Reagent
- 2.5 mL Combitips® or equivalent for Precision Dispenser
- 1% Sodium Hypochlorite Solution (20% household bleach)

Perform the probe decontamination procedure with 1% sodium hypochlorite solution before each Tacrolimus II assay run and when switching from Tacrolimus II to any other IMx assay. For further information refer to your IMx System Operation Manual, Section 9.

The list of required materials and the procedure to perform an IMx Tacrolimus II Calibration or MODE 1 Assay can be found in your IMx System Operation Manual, Section 5.

Do not mix different lots of reaction cells within a run.

The IMx Tacrolimus II assay requires a minimum volume of 200 mL of MEIA #2 Diluent Buffer in the buffer bottle in order to properly process an assay run. Before initiating an IMx Tacrolimus II assay, visually check that at least 200 mL of MEIA #2 Diluent Buffer is present. Do not add diluent buffer to the buffer bottle or switch buffer bottles during an assay run.

The IMx Tacrolimus II assay requires a manual pretreatment step of all whole blood samples, MODE 1 Calibrator, Calibrators and Controls.

NOTE: If a specimen requires dilution, it must be diluted prior to the manual pretreatment step. Refer to the DILUTION INFORMATION section in this package insert.

Manual Pretreatment Step

Attention: To obtain optimal results for the IMx Tacrolimus II assay the Manual Pretreatment Steps listed below must be followed precisely. **Special attention must be paid to the pipetting of the whole blood (step 2), the pipetting of the precipitation reagent (step 3), and the vortex step (step 4).** Symptoms of inadequate pipetting or vortexing could include calibration curve failures, erratic control values, decreased sensitivity or poor precision.

1. Mix each sample, MODE 1 Calibrator, Calibrator or Control thoroughly.
2. Immediately after mixing, precision pipet 150 µL of each sample, MODE 1 Calibrator, Calibrator or Control into an X-SYSTEMS Centrifuge Tube.
   Note: A new pipet tip must be used each time 150 µL is aspirated.
3. Set the Precision Dispenser to dispense 150 µL and fill it with Tacrolimus II Whole Blood Precipitation Reagent. Purge the syringe of air bubbles by dispensing into a suitable waste container. Dispense 150 µL of Precipitation Reagent into each centrifuge tube by touching the end of the dispensing syringe tip to the wall of the centrifuge tube and depressing the button. Immediately cap the centrifuge tube.
4. Vortex each tube vigorously at the maximum vortex setting for a minimum of 10 seconds. **It is critical that a homogeneous mixture is obtained. No unmixed portion of the mixture should be present at the bottom of the centrifuge tube.**

   Vortexing should be performed immediately after the addition of the precipitation reagent to minimize the time needed to break up any pellets that may form. Not all vortex mixers may provide adequate mixing; visual inspection is required.
5. VISUALLY INSPECT EACH TUBE TO INSURE NO UNMIXED SAMPLE REMAINS IN THE BOTTOM OF THE TUBE. If unmixed sample remains in the bottom of the tube, dislodge by inverting and tapping the bottom of the tube, then revortex the sample.
6. Load each centrifuge tube into an X-SYSTEMS Centrifuge taking care to balance the rotor. Only an even number of tubes can be centrifuged at one time. A balance tube can be added if necessary.
7. Centrifuge the tubes for 4 minutes.
8. Uncap the tubes and **decant** the supernatant into the sample well of an IMx reaction cell. **Do not disturb the pellet.**

   Note: Samples should not be left in the centrifuge tubes for more than 10 minutes following centrifugation. Once the samples are decanted into the IMx reaction cell, the carousel should be run **immediately** to prevent evaporation.

DILUTION INFORMATION

SAMPLE DILUTION PROCEDURE

Manual Dilution Protocol

Patient specimens with tacrolimus concentrations reported as greater than 30 ng/mL may be diluted up to 1:4 using the IMx Tacrolimus II MODE 1 Calibrator (0 ng/mL). Specimens must be diluted **BEFORE** being treated with the IMx Tacrolimus II Whole Blood Precipitation Reagent.

The concentration reported by the IMx system must be multiplied by the manual dilution factor to obtain the final sample concentration.

$$\text{Final Sample Concentration} = \text{Reported Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Sample} + \text{Volume of Dilution Reagent})}{\text{Volume of Sample}}$$

Manual Dilution

The dilution should be made so that the diluted specimen reads above the IMx Tacrolimus II B Calibrator on the calibration curve.

QUALITY CONTROL PROCEDURES

CALIBRATION

For an IMx Tacrolimus II calibration, run all IMx Tacrolimus II Calibrator levels in duplicate in the first 12 carousel positions. All levels of controls must be processed as a means of evaluating the

calibration curve. The RESULTS section below provides an explanation of the type of curve fit used by the IMx® Tacrolimus II assay and the assay-specific checks that are used to evaluate the acceptability of the curve.

Once the assay calibration is accepted and stored, all subsequent runs are tested in MODE 1 with the MODE 1 Calibrator in positions 1 and 2 of the carousel.

Refer to the IMx System Operation Manual, Section 5 for:

- Setting up an assay calibration run
- When recalibration may be necessary
- System and Operator Verification
- MEIA Calibration and MEIA MODE 1 Assay Test Results Tape Explanation

QUALITY CONTROL

The minimum control requirement for an IMx Tacrolimus II MODE 1 assay is **one** control on each carousel. All levels of controls should be processed at least one time during each 8 hour shift. If the quality control procedures in your laboratory require more frequent use of controls, follow those procedures. When a new lot of the IMx Tacrolimus II Reagent Pack is used, run all levels of IMx Tacrolimus II Controls.

IMx Tacrolimus II Control values must be within the range specified in the **REAGENTS** section of this package insert. If a control value is out of its specified range, the test results may be invalid and assay recalibration may be indicated. Refer to the IMx System Operation Manual, Section 10 for a description of troubleshooting procedures.

RESULTS

The IMx Tacrolimus II assay utilizes a four parameter logistic curve fit (4 PLC) to generate a calibration curve. The following are assay-specific checks used to evaluate the calibration curve:

Assay Parameters	Calibrator Evaluation (AVGR)
MIN CHECK 1	Calibrator B/Calibrator A
MAX CHECK 1	Calibrator B/Calibrator A
MIN CHECK 3	Calibrator D/Calibrator A
MAX CHECK 3	Calibrator D/Calibrator A
MIN CHECK 5	Calibrator F/Calibrator A
MAX CHECK 5	Calibrator F/Calibrator A

The operator must confirm that the following parameters fall within the acceptable ranges:

RERR (Rate Error)	RMSE (Root Mean Square Error)
±20	≤10

FLAGGED RESULTS

For a description of the flags that appear in the NOTE column on the test results tape, refer to your IMx System Operation Manual, Section 5.

LIMITATION OF THE PROCEDURE

As with all analyte determinations, the tacrolimus value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures. The concentration of tacrolimus in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The immunoassays are nonspecific and cross react with metabolites. When elimination of tacrolimus is impaired (e.g. during cholestasis), tacrolimus metabolites may accumulate. The immunoassay may overestimate the concentration of tacrolimus. In such cases, the use of a specific assay (e.g. HPLC/MS/MS) could be considered.

Specimens with a tacrolimus value exceeding 30 ng/mL (HIGH RANGE, assay parameter 112.28) are flagged with the code ">30". To quantitate the concentration of these specimens, perform the manual dilution procedure. Refer to the **DILUTION INFORMATION** Section in this package insert.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which utilize mouse monoclonal antibodies.[25,26] These specimens should not be assayed with the IMx Tacrolimus II assay.

EXPECTED VALUES

CAUTION:
The complexity of the clinical state and individual differences in sensitivity to the immunosuppressive effects of tacrolimus will cause different requirements for optimal whole blood levels of tacrolimus. Each patient should be thoroughly evaluated clinically before treatment adjustments are made. The physician should establish individual patient ranges based on these clinical evaluations. Individual whole blood tacrolimus values cannot be used as the sole indicator for making changes in the treatment regime.

An IMx Tacrolimus II clinical study evaluated 102 allograft liver transplant patients for a period of three months post-transplant. A total of 2604 tacrolimus concentrations from these 102 patients, ranging from 1.5 to 43.9 ng/mL, was measured using the IMx Tacrolimus II assay. The clinical data demonstrated that there was not a clear relationship between the tacrolimus dosing, whole blood concentrations of tacrolimus and adverse events of rejection and nephrotoxicity. This was most evident during the initial three weeks post-transplant when the frequency of adverse events was greatest and the pharmacokinetic profile of tacrolimus was the most variable. The greatest frequency of adverse events, 51 of 99 (51.6%), occurred during the initial three weeks - 34 of 53 (64.2%) rejection events and 17 of 46 (37.0%) nephrotoxic events.

Following the initial three weeks post-transplant, the number of adverse events declined. This decline in adverse events occurred even though the mean total daily dose of tacrolimus and the mean tacrolimus whole blood concentrations remained relatively constant in the study population over the three months post-transplant.

The Consensus Document has reported that the therapeutic range of tacrolimus is not clearly defined, but target 12-h trough whole blood concentrations are 5-20 ng/mL early post-transplant. Higher concentrations are associated with an increase incidence of adverse effects. Twenty-four h trough concentrations are 33-50% less than the corresponding 12-h trough levels.[1]

SPECIFIC PERFORMANCE CHARACTERISTICS

SPECIFICITY

Purified tacrolimus metabolites are not commercially available for cross-reactivity testing.

Physiological concentrations of the tacrolimus metabolites in whole blood and the clinical significance of the metabolites have not been defined.

Metabolites:

Test Compound	Test Conc.	Observed Conc. Mean (n = 6)	Percent (%) Cross-reactivity
M-I	10 ng/mL	<1.5 ng/mL	N/A*
M-II	10 ng/mL	5.4 ng/mL	54.0
M-III	10 ng/mL	6.7 ng/mL	67.0
M-IV	10 ng/mL	<1.5 ng/mL	N/A*
M-V	10 ng/mL	6.2 ng/mL	62.0
M-VI	10 ng/mL	<1.5 ng/mL	N/A*
M-VII	10 ng/mL	<1.5 ng/mL	N/A*
M-VIII	10 ng/mL	<1.5 ng/mL	N/A*

* Cross-reactivity could not be determined for concentrations less than analytical sensitivity.

Tacrolimus metabolites M-II, M-III and M-V were tested at 10 ng/mL in the presence of 11 ng/mL tacrolimus. Metabolite M-II resulted in a doubling of the tacrolimus concentration while M-III and M-V resulted in a 20-40% increase in the tacrolimus concentration.

Cross-reactivity was tested for compounds whose chemical structure or concurrent usage could cause potential interference with the IMx® Tacrolimus II assay. The following compounds were tested at the stated concentrations. These compounds yielded mean concentrations (n = 5) less than the analytical sensitivity of the assay (1.5 ng/mL).

Test Compound	Test Conc.	Test Compound	Test Conc.
N-Acetylprocainamide	100 µg/mL	Kanamycin	100 µg/mL
Acyclovir	1000 µg/mL	Lidocaine	100 µg/mL
Amikacin	100 µg/mL	Mycophenolic Acid	200 µg/mL
Amphotericin B	100 µg/mL	Penicillin	100 µg/mL
Azathioprine	100 µg/mL	Phenytoin	100 µg/mL
Carbamazepine	100 µg/mL	Prazosin	25 µg/mL
Cephalosporine	100 µg/mL	Prednisolone	100 µg/mL
Chloramphenicol	100 µg/mL	Prednisone	100 µg/mL
Cimetidine	100 µg/mL	Primidone	100 µg/mL
Cyclosporine	1000 ng/mL	Procainamide	100 µg/mL
Digitoxin	100 ng/mL	Quinidine	100 µg/mL
Digoxin	10 ng/mL	Rifampin	100 µg/mL
Disopyramide	5 µg/mL	Spectinomycin	100 µg/mL
Erythromycin	100 µg/mL	Valproic Acid	1000 µg/mL
Furosemide	100 µg/mL	Vancomycin	100 µg/mL
Ganciclovir	1000 µg/mL	Verapamil	10 µg/mL

Cross-reactivity was also tested in the presence of 11 ng/mL tacrolimus for azathioprine (1 µg/mL), cyclosporine (200 ng/mL), erythromycin (1 µg/mL), prednisone (10 µg/mL), and mycophenolic acid (200 µg/mL). These compounds showed no apparent change in concentration (less than 1.5 ng/mL incremental change).

INTERFERING SUBSTANCES

The compounds listed below, when tested with the IMx Tacrolimus I assay at the spiked concentrations indicated below, resulted in les than 12% error in detecting tacrolimus.

Compound	Concentration Tested
Bilirubin	40 mg/dL
Protein	25 g/dL
Triglycerides	800 mg/dL
Uric Acid	40 mg/dL

Clinical specimens containing endogenous levels of cholesterol up t 366 mg/dL, triglycerides up to 988 mg/dL and bilirubin up t 33.4 mg/dL, resulted in less than 15% error in detecting tacrolimus.

Hematocrit values ranging from 25 to 45% resulted in less than 12% error in detecting tacrolimus.

SENSITIVITY

Functional

The functional sensitivity of the IMx Tacrolimus II assay was deter mined by serially diluting 25 tacrolimus patient samples with th MODE 1 Calibrator and determining where the observed value fall out of the ±20% tolerance limits. The functional sensitivity range from analytical sensitivity to 4.1 ng/mL.

This range was confirmed by serially diluting five patient sample using drug free whole blood as the patient sample diluent.

Analytical

The analytical sensitivity was determined using a non-parametri statistical method. At the lower 2.5 percentile, the sensitivity or lowes detection level is 1.5 ng/mL.

PRECISION

Precision was determined as described in National Committee fo Clinical Laboratory Standards (NCCLS) protocol EP5-T2[27] using processed human whole blood with 5, 11, and 22 ng/mL of tacrolimus added. Results from these studies typically yielded CV's of less than 15%. The following are representative results from pooled data using three reagent lots tested on ten instruments.

Sample	n	Mean (ng/mL)	Within Run SD	Within Run CV%	Between Day SD	Between Day CV%	Total SD	Total CV%
1	800	5.02	0.44	8.7	0.08	1.7	0.60	12.0
2	800	10.89	0.57	5.3	0.19	1.8	0.86	7.9
3	800	21.42	0.87	4.1	0.60	2.8	1.41	6.6

Additional NCCLS studies were done using processed human whole blood with 2 and 3 ng/mL tacrolimus added. The following are pooled estimates of precision data using three reagent lots tested on six instruments.

Sample	n	Mean (ng/mL)	Within Run SD	Within Run CV%	Between Day SD	Between Day CV%	Total SD	Total CV%
1	480	2.36	0.29	12.4	0.10	4.1	0.39	16.3
2	480	3.36	0.32	9.6	0.12	3.6	0.39	11.5

RECOVERY

Known amounts of tacrolimus were added to drug-free whole blood. The concentration of tacrolimus was determined using the IMx® Tacrolimus II assay and the resulting percent recovery was calculated.

Theoretical (ng/mL)	Observed (ng/mL)	n**	Recovery* (%)	Range (%)
2.0	2.3	40	115.0	(70.0-145.0)
3.0	2.8	24	93.3	(80.0-106.0)
6.0	5.8	12	96.7	(90.0-101.7)
12.0	10.5	7	87.5	(81.7-90.8)
20.0	17.9	5	89.5	(87.0-95.0)
25.0	23.8	4	95.2	(93.2-97.2)

Average % Recovery = 96.2%

$$*\%\text{Recovery} = \frac{\text{Observed } (^{ng}/_{mL})}{\text{Theoretical } (^{ng}/_{mL})} \times 100$$

** Number of replicates for 95% confidence level based on 10% tolerance limit at each concentration.

ACCURACY BY CORRELATION

The Abbott IMx Tacrolimus II assay was compared to a HPLC/MS/MS method. The results of the specimen testing are shown below.

Methodology	Number of Observations	Intercept	Slope	Correlation Coefficient
IMx Tacrolimus II vs. HPLC/MS/MS	105	0.63 (0.24, 1.03)*	0.94 (0.91, 0.97)*	0.987

* 95% confidence interval for slope and intercept

Sample Range (IMx) 3.7 - 24.3 ng/mL.

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